

IN THE CLAIMS:

1-38. (Canceled)

39. (Currently amended) A method of inducing somatic differentiation of human embryonic stem (hES) cells *in vitro* into human neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation to a cell selected from a group consisting of neurons, oligodendrocytes and an astrocytes identified by the expression of at least one of NCAM, nestin, vimentin or Pax-6, and by the lack of expression of Oct-4, said method comprising:

obtaining undifferentiated human pluripotent embryonic stem hES cells ; and
~~providing~~culturing the hES cells under a controlled differentiating condition which is non-permissive for stem cell renewal, does not kill cells or induce unidirectional differentiation toward extraembryonic lineages to induce somatic differentiation of the hES cells.

40. (Currently amended) The method according to claim 39 wherein said undifferentiated pluripotent ~~embryonic stem cell is~~ hES cells are capable of proliferation *in vitro* and differentiation to neural progenitor cells, neuron cells, oligodendrocytes and astrocytes, ~~or glial cells~~ and is are immunoreactive with markers for human pluripotent stem cells including SSEA-4, GCTM-2 antigen, and TRA 1-60.

41. (Currently amended) The method according to claim 39 wherein said undifferentiated pluripotent ~~embryonic stem~~hES cells express Oct-4.

42. (Currently amended) The method according to claim 39 wherein said undifferentiated pluripotent ~~embryonic stem~~hES cells maintain a diploid karyotype during prolonged cultivation *in vitro*.

43. (Currently amended) The method according to claim 39 wherein said undifferentiated pluripotent ~~embryonic stem~~hES cells form tumors when injected in the testis of immunodeprived SCID mice.

44. (Currently amended) The method according to claim 39 wherein said undifferentiated ~~human~~ pluripotent ~~embryonic stem~~hES cells are prepared according to a method comprising:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from the embryo;

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; and
recovering stem cells.

45. (Currently amended) The method according to claim 44 wherein said undifferentiated ~~human pluripotent embryonic stem~~ hES cells are prepared further comprising:

culturing the ICM cells on a fibroblast feeder layer to promote proliferation of embryonic stem cells prior to recovering the stem cells from the feeder layer, wherein the fibroblast feeder cells are arrested in their growth.

replating the stem cells from the fibroblast feeder layer onto another fibroblast feeder layer; and

culturing the stem cells for a period sufficient to promote proliferation of morphologically undifferentiated stem cells.

46. (Previously presented) The method according to claim 39 wherein the conditions for inducing somatic differentiation of stem cells are selected from any one of the following including:

culturing the undifferentiated stem cells for prolonged periods and at high density on a fibroblast feeder cell layer to induce differentiation;

culturing the undifferentiated stem cells in serum free media;

culturing the undifferentiated stem cells on a differentiation inducing fibroblast feeder layer and wherein said fibroblast feeder layer does not induce extra embryonic differentiation and cell death;

culturing to a high density in monolayer or on semi-permeable membranes so as to create structures mimicking the postimplantation phase of human development; or

culturing in the presence of a chemical differentiation factor selected from the group including bone morphogenic protein-2 or antagonists thereof.

47-50. (Canceled)

51. (Currently amended) A method of inducing differentiation of neural progenitors to somatic cells, said method comprising:

obtaining a source of neural progenitor cells derived from human pluripotent embryonic stem cells *in vitro* wherein the neural progenitor cells are capable of further differentiation to a cell selected from the group consisting of neurons, oligodendrocytes and astrocytes;

culturing the neural progenitor cells on an adhesive substrate in the presence of a serum free media and growth factors; and

inducing the neural progenitor cells to differentiate by withdrawal of the growth factors.

52-55. (Canceled)

56. (Currently amended) A method of inducing differentiation of neural progenitors to somatic cells, wherein said progenitors are derived from human pluripotent embryonic stem cells *in vitro*, said method comprising:

obtaining a source of neural progenitor cells, wherein said neural progenitor cells are derived from human pluripotent embryonic stem cells *in vitro* and are capable of further differentiation ~~differentiating~~ into a cell selected from the group consisting of ~~neuron~~neurons, cells or glial cellsoligodendrocytes and astrocytes; and

culturing the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and laminin in the presence of a serum free media; and[[.]]

inducing the neural progenitor cells to differentiate to somatic cells under conditions which favor somatic differentiation.

57. (Original) The method according to claim 56 wherein the cells are further cultured in the presence of retinoic acid.

58. (Previously presented) The method according to claim 56 or 57 wherein said somatic cells are neurons.

59. (Canceled)

60. (Currently amended) A method of inducing differentiation of neural progenitors to somatic cells, said method comprising:

obtaining a source of neural progenitor cells, wherein said neural progenitor cells are derived from human pluripotent embryonic stem cells *in vitro* and are capable of further differentiation differentiating into a cell selected from the group consisting of ~~neuron~~neurons, cells or glial cells oligodendrocytes and astrocytes;

culturing the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and fibronectin, wherein the neural progenitor cells are cultured before and after plating on poly-D-lysine and fibronectin in serum free medium in the presence of PDGF-AA and bFGF; and

inducing the neural progenitor cells to differentiate to somatic cells under conditions which favor somatic differentiation.

61. (Previously presented) The method according to claim 60 wherein the progenitor cells are cultured after plating on said adhesive substrate in the presence of PDGF-AA, basic FGF and EGF.

62. (Previously presented) The method according to claim 61 further including culturing the neural progenitor cells after plating on said adhesive substrate in the presence of T3.

63. (Currently amended) The method according to claim ~~62~~60 wherein said somatic cells induced are ~~glial cells~~oligodendrocytes or astrocytes.

64. (Currently amended) A method of producing an enriched preparation of human pluripotent ES cell derived neural progenitor cells wherein the neural progenitor cells are capable of further differentiation to a cell selected from the group consisting of neurons, oligodendrocytes and astrocytes, said method comprising:

obtaining undifferentiated human embryonic stem cells comprising obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development; removing inner cells mass (ICM) cells from the embryo; culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; recovering undifferentiated stem cells;

inducing somatic differentiation of the undifferentiated embryonic stem cells to the neural progenitor cells by providing differentiating conditions which are non-permissive for stem cell renewal, do not kill cells or induce unidirectional differentiation toward extraembryonic lineages;

identifying ~~athe~~ neural progenitor cells by expressed markers selected from the group consisting of polysialyated N-CAM, an intermediate filament protein~~nestin~~, ~~vimentin~~ and the transcription factor Pax-6; and

culturing the neural progenitor cells to promote proliferation and propagation.

65. (Currently amended) The method according to claim 64 wherein the differentiating conditions comprise culturing the neural progenitor cells in serum free medium comprising DMEM/F12 supplemented with growth factors.

66. (Original) The method according to claim 65 wherein the growth factors include B27, EGF and bFGF.

67. (Previously Presented) The method according to claim 66 including further culturing to eliminate non-neural cells, said further culturing comprising selective culturing in serum free media including DMEM/F12 supplemented with growth factors.

68. (Original) The method according to claim 67 wherein the further culturing includes the transfer of undifferentiated ES cell clumps into serum free medium comprised of DMEM/F12 supplemented with B27, bFGF and EGF and cultivation of the resulting neural progenitors as spheres or monolayers.

69-85. (Canceled)

86. (Previously presented) The method according to claim 58 wherein said neurons are mature neurons.

87. (Canceled)

88. (Currently amended) A method of inducing somatic differentiation of human embryonic stem cells *in vitro* into neural progenitor cells wherein the neural progenitor cells are capable of further differentiation to a cell selected from the group consisting of neurons, oligodendrocytes and astrocytes, said method comprising:

- obtaining undifferentiated human pluripotent embryonic stem cells;
- culturing the undifferentiated human pluripotent embryonic stem cells on a fibroblast feeder cell layer for a prolonged period of time and at high density sufficient to induce differentiation;
- further culturing the cells in serum free media thereby obtaining said neural progenitor cells.

89. (Currently amended) A method of producing an enriched preparation of human neural progenitor cells wherein the neural progenitor cells are capable of further differentiation to a cell selected from the group consisting of neurons, oligodendrocytes and astrocytes, said method comprising:

- obtaining human pluripotent embryonic stem cells;
- culturing the human pluripotent embryonic stem cells on a fibroblast feeder cell layer to induce differentiation;
- further culturing the stem cells in serum free media supplemented with at least one growth factor;
- identifying the neural progenitor cells by the expression of at least one of expressed markers of primitive neuroectoderm and neural stem cells, intermediate filament proteins or the transcription factor Pax-6; and
- culturing the neural progenitor cells to promote proliferation and propagation.

90. (Currently amended) The method of claim 89, wherein the human pluripotent embryonic cells are obtained by

- obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;
- removing inner cells mass (ICM) cells from the embryo; and

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells.

91. (Previously presented) The method of claim 89, wherein said growth factor is selected from B27, EGF or bFGF.

92. (Currently amended) The method of claim 89, wherein the neural progenitor cells are identified by the expression of at least one of polysialyated N-CAM, nestin, or vimentin, ~~or Pax-6~~.

93. (Previously presented) The method of claim 89, wherein the neural progenitor cells are cultured in serum free media supplemented with at least one growth factor to promote proliferation and propagation.

94. (Previously presented) The method of claim 89, wherein the neural progenitor cells are cultured as monolayers or spheres.

95. (Currently amended) A method of inducing somatic differentiation of human embryonic stem cells *in vitro* into neural progenitor cells wherein the neural progenitor cells are capable of further differentiation to a cell selected from the group consisting of neurons, oligodendrocytes and astrocytes, said method comprising;

obtaining undifferentiated human pluripotent embryonic stem cells; and
culturing the undifferentiated stem cells in serum free media.

96. (New) A method according to claim 39 wherein the controlled condition for inducing somatic differentiation of embryonic stem cells into neural progenitor cells consists of culturing the undifferentiated stem cells for prolonged periods and at high density on a fibroblast feeder cell layer.

97. (New) A method according to claim 39 wherein the controlled condition for inducing somatic differentiation of embryonic stem cells into neural progenitor cells consists of culturing the undifferentiated stem cells in serum free media.

98. (New) A method according to claim 39 wherein the controlled condition for inducing somatic differentiation of embryonic stem cells into neural progenitor cells consists of culturing the undifferentiated stem cells for prolonged periods and at high density on a fibroblast feeder cell layer and in serum free media.
99. (New) A method according to claim 56 wherein the conditions which favor somatic differentiation consist of withdrawal of growth factors.